

AD \_\_\_\_\_

Award Number: DAMD17-99-1-9321

TITLE: Oxidized DNA Bases in Breast Cancer

PRINCIPAL INVESTIGATOR: Mehul Desai, Ph.D.

CONTRACTING ORGANIZATION: New York University School of Medicine  
New York, New York 10016

REPORT DATE: August 2000

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 074-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE August 2000	3. REPORT TYPE AND DATES COVERED Annual Summary ( 08/01/99 - 07/31/00)		
4. TITLE AND SUBTITLE Oxidized DNA Bases in Breast Cancer		5. FUNDING NUMBERS DAMD17-99-1-9321		
6. AUTHOR(S) Mehul Desai, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) New York University School of Medicine New York, New York 10016  E-MAIL: mehul.desai@med.nyu.edu		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSORING / MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words)  5-Hydroxymethyl-2'-deoxyuridine (HMdU), an oxidized DNA base, has been proposed as a biomarker of breast cancer risk, but there are no facile assays to measure HMdU. We have developed a simple and sensitive fluorometric method for HMdU quantitation, which should be useful in the epidemiological studies. This method utilizes 7-dimethylamino-coumarin-3-acetic-acid (DMACA) as the fluorescent probe for the post-column HMdU derivatization. DMACA postlabeling of HMdU results in the formation of two baseline-separated products, while reaction with dU or dT results in a single fluorescent product. This indicates that post-labeling occurs on primary alcohols. Mass spectral analysis suggests that dT*, dU* and HMdU*I products contain the fluorophore on the 5'-CH <sub>2</sub> OH group (sugar moiety), while HMdU*II on the 5-CH <sub>2</sub> OH of the base. The product quantitation is linear within the range of 2 pmole to 5 fmole, while limit of detection is about 0.01 fmole. Further physiochemical characterization of the products is underway. To quantitate HMdU, cellular DNA will be enzymatically digested to nucleosides, which are well separated by reverse-phase HPLC-1. HMdU-containing fractions are concentrated, subjected to coumarin post-labeling and quantitated using normal phase HPLC-2 with fluorescence detection at $\lambda_{ex}$ = 380 nm and $\lambda_{em}$ = 460 nm. We are in the process of method validation.				
14. SUBJECT TERMS HMdU Post-labeling, Fluorescent labeling, HMdU, Breast Cancer			15. NUMBER OF PAGES 10	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

## Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4-8
Key Research Accomplishments.....	9
Reportable Outcomes.....	9
Conclusions.....	9
References.....	N/A
Appendices.....	10

## 1. Introduction:

Oxidative DNA damage induced by oxidative stress is increasingly considered as a major factor associated with the breast cancer. The oxidative stress has been shown to cause both DNA strand breaks and modification of DNA bases. Our hypothesis is that the oxidative DNA damage begins much earlier than the clinical manifestation of breast cancer. Recent findings showed significantly higher levels of 5-hydroxymethyl-2'-deoxyuridine (HMdU), an oxidized DNA base, in patients with breast cancer and those at high risk of this disease. HMdU is also proposed to be a biomarker of breast cancer risk. This hypothesis is further supported by the recent finding of our laboratory which showed that sera of healthy women with a family history of breast cancer and women diagnosed with breast cancer several years after blood donation had elevated amounts of anti-HMdU antibodies. This suggests that in the early stages of cancer development cells are under oxidative stress, which is manifested by significant HMdU formation. To test our hypothesis, it would be important to quantitate HMdU levels in DNA of WBC of a large group of women to assess the oxidative stress and to determine the oxidative DNA damage before the clinical diagnosis of breast cancer is possible. Although methods are available for detection and quantitation of HMdU from various biological samples, but either it are expensive (GC/MS) or have problem with radioactive waste disposal (tritium labeling). In present study we are developing the simple, sensitive and cost effective fluorescent-labeling method for quantitation of HMdU. Once the method is developed it will be validated and will be utilized to detect and quantitate HMdU from cell samples.

## 2. Studies and Results

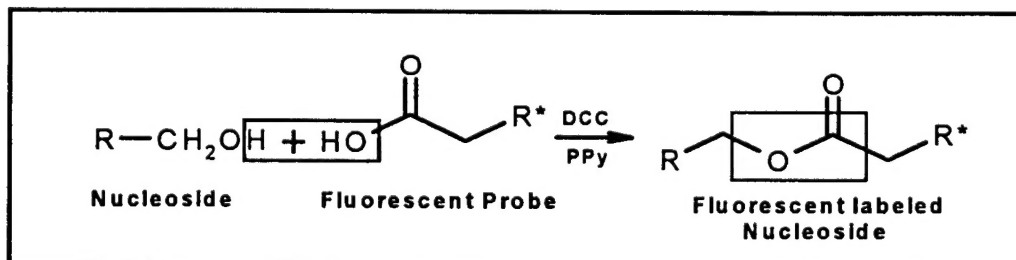
### 2.a. Change in the Fluorescence Post-labeling protocol:

We had proposed to post-label HMdU with 7-methoxycoumarin-3-carbonyl azide (Molecular Probes) in tetrahydrofuran (THF) under anhydrous conditions overnight at the elevated temperature. In the process of method development, it was observed that higher losses occur in labeling of small amounts of HMdU due to the trace amounts of moisture present in the environment, which competes with the low level of HMdU. Moreover, stringent conditions required for the post-labeling would be more difficult to follow by other laboratories. Therefore, the protocol for the fluorescence post-labeling of 5-hydroxymethyl-2'-deoxyuridine (HMdU) has been developed.

In search for better fluorescent reagent for simple and sensitive post-labeling method, we found that 7-dimethyl-amino-coumarin-4-acetic acid (DMACA), which reacts with HMdU in the presence of a catalyst and a linker to form labeled product, fulfils those requirements.

### 2.b. New HMdU Post-labeling Method:

This method uses the esterification reaction for the fluorescence labeling of HMdU with 7-dimethyl-amino-coumarin-4-acetic acid (DMACA, Molecular probes) in the presence of a catalyst 4-pyrrolidino-pyridine (PPy) and 4,4'-dicyclohexyl-carbodiimide (DCC). General reaction can be given as:

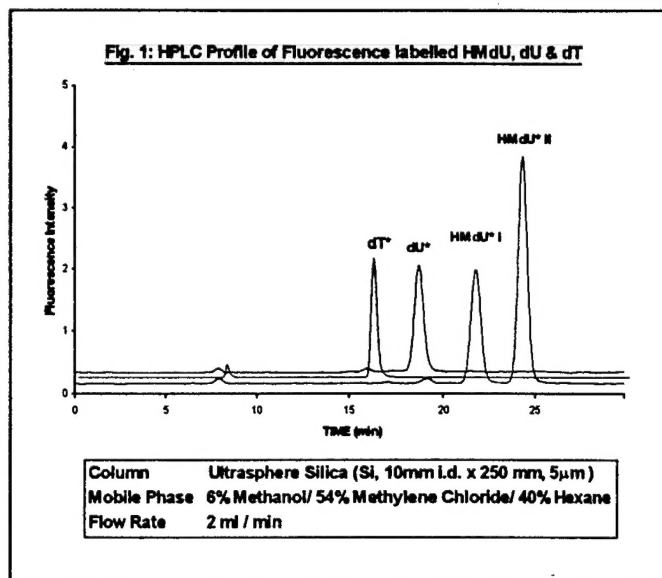


**Post-labeling Method:**

HMdU (1 nmole) was suspended in anhydrous acetonitrile (ACN) in a polypropylene eppendorff tube and DMACA (100 nmole) reagent and PPy (5 nmole) were added. The reaction mixture was mixed well, DCC (1 $\mu$ mole) in anhydrous ACN was added, and heated at 65-70°C in a heating block for 2 h. The heating block is covered with aluminum foil in order to avoid the photo-degradation of the reagent at an elevated temperature.

After the reaction was over, the reaction mixture was dried. Fluorescent HMdU products were isolated using silica-gel Sep-Pack (5% methanol/95% methylene chlo-ride), and further, purified using normal phase HPLC. Reaction with HMdU resulted in the formation of two baseline separated fluorescent products HMdU\*-I and HMdU\*-II (Fig. 1).

Similarly, 2'-deoxyuridine and thymidine were labeled using DMACA in the presence of PPy and DCC. Both dU and dT formed fluorescent coumarin products dU\* and dT\*, which is shown in Fig. 1.



Upon fluorescence labeling, dU and dT gives a single fluorescence labeled product each, while HMdU gives two fluorescence products, which suggests that the labeling occurs on the primary hydroxyl m (-CH<sub>2</sub>OH) groups of the 2'-deoxynucleosides. Further, the ammonolysis of HMdU\*1, dU\* & dT\* released respective nucleosides, as determined by HPLC analysis of the ammonolysed products, but that of HMdU\* II did not. This suggests that HMdU\* II labeling has occurred on the primary hydroxyl group (-CH<sub>2</sub>OH) on the 5-position of the base moiety. This was further confirmed by GC/MS analysis of the hydrolysis products.

Mass spectral analysis of labeled nucleoside products suggests that all fluorescent nucleoside-coumarin derivatives are mono-labeled. Mass spectra of HMdU\*1, dU\* & dT\* show the m/z = 347 mass peak, this (M+1) fraction can be assigned to the sugar moiety attached to the label [247.2 (DMACA) + 117 (2'-deoxy- ribose) - 18 (H<sub>2</sub>O) = 346.2 ], while HMdU\* II does not show that mass signal. Further physiochemical characterization of the products is currently underway. The spectral data for the fluorescent-labeled nucleosides are shown in Table 1.

**Table 1 : Spectral Data of Coumarin-labeled Nucleosides**

Nucleoside/ coumarin Product	UV $\lambda_{\max}$ (nm)	FLUORESCENCE		Recovery of Nucleoside after hydrolysis
		Excitation $\lambda_{\max}$	Emission $\lambda_{\max}$	
dU *	372	385	448	Yes
dT *	373	385	446	Yes
HMdU* I	383	380	460	Yes
HMdU* II	385	380	455	No

**2.c. Method Development for the Detection and Quantitation of HMdU****HPLC Method:**

The normal phase HPLC method was developed for the detection and quantitation of labeled HMdU products for better sensitivity, as compared to the reverse-phase HPLC. Fluorescent HMdU products are analyzed using semi-preparative silicagel column (Ultrasphere, Beckman, 250 mm x 10 mm, 5  $\mu$ m) using fluorescence detector (FP-920, Jasco Inc.,) with  $\lambda$  excitation = 380 nm and  $\lambda$  emission = 460 nm. HMdU-coumarin products are eluted isocratically at 2 ml/min flow-rate, with 8% methanol/42% dichloromethane/50% n-hexane as the eluent, at 21.7 min and 24.3 min retention times for HMdU\*-I and HMdU\*-II, respectively.

**Pre-purification of the Coumarin-labeled Products Prior to HPLC Analysis:**

A pre-purification method has been developed for a better resolution and separation of the coumarin-labeled 2'-deoxyribonucleoside products, as well as to prolong the useful life of the HPLC column. Pre-purification removes side products of the reagent with catalyst and linker in an earlier fraction, while the products of interest elute in the last fraction. Silica and octadecylsilane (ODS) columns were tried for this purpose. Silica SPE was found to be more suitable for the pre-purification.

The dried reaction mixture is reconstituted in Solvent A (3% methanol/37% dichloromethane/ 60% n-hexane) and placed on the top of a pre-conditioned SPE Silica cartridge (500 mg, 3 ml, Accubond, J&W Scientific). It is followed by elution with 6 ml of solvent A, then 3 ml of Solvent B (5% methanol/95% dichloromethane), and finally, 5 ml Solvent C (15% methanol/95% dichloromethane). The HMdU-DMACA as well as dU\* fluorescent products are in the last fraction. The last fraction is dried and analyzed by HPLC for the quantitation of labeled 2'-deoxyribonucleosides.

**Optimization of the Post-labeling Reaction:**

The conditions for HMdU post-labeling reaction were optimized for the maximum yield of the HMdU/coumarin product by changing one parameter at a time or in combination. The reaction conditions were optimized at 1 nmole HMdU level by changing parameters viz. amount of the reagent DMACA, catalyst PPy, linker DCC, reaction time, and reaction volume.

**Effect of DMACA:** The HMdU post-labeling reaction was carried out with various ratios of HMdU to DMACA reagent ranging from 1:1 to 1:200 nmoles, while keeping other parameters constant. It was found that the optimal yield was obtained with 1:100 (HMdU:DMACA).

**Effect of PPy :** The ratio of PPy catalyst to HMdU was also optimized. The HMdU post-labeling reaction was carried out with various ratios of HMdU to PPy ranging from 1:0.01 to 1:200 nmoles, while keeping DMACA constant at 100 nmoles. It was found that the optimal yield was obtained with 1 HMdU:5 PPy. It was evident from the graph that the amount of catalyst plays a role in the labeling of HMdU, with the increase in the amount of catalyst yielding decreased levels of labeled products.

**Effect of DCC :** The HMdU post-labeling reaction was carried out with various ratios of HMdU to the linker DCC ranging from 500 nmoles to 5  $\mu$ moles per 1 nmole HMdU, while holding constant DMACA at 100 nmoles and PPy at 5 nmoles. It was found that the optimal yield was obtained with 1 nmole HMdU: 5  $\mu$ moles DCC.

**Reaction Time :** After optimizing the reagent variables, the time function of the HMdU post-labeling was also optimized. Yields of the HMdU products were determined at different times ranging from 15 min to



2 h, in 15 min intervals. The maximum yield was obtained within 45 min heating time. Hence, the time factor was set at 1 h from the start to the end of the reaction.

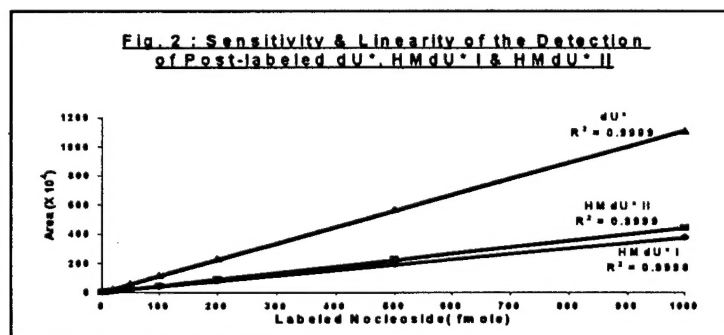
**Effect of Reaction Volume :** Different reaction volumes (before adding DCC) ranging from 200  $\mu$ l to 900  $\mu$ l was utilized, while keeping other parameters at the optimal levels. It was observed that with an increase in the reaction volume, the yield of labeled HMdU products decreased, with the optimal yields obtained at 200 to 300  $\mu$ l. These results suggest that the post-labeling reaction is reagent concentration-dependent.

#### 2.d. Reporter Molecule:

In order to know the losses in the yield of labeled HMdU during the post-labeling reaction a reporter molecule is being introduced. This reporter molecule undergoes the same post-labeling conditions as HMdU. The yield of this fluorescence-labeled reporter molecule will allow us to determine the extent of loss in the labeling reaction. For this reason we have decided to add dU (5 pmole) to each post-labeling reaction, product of which (dU\*) elutes before labeled HMdU products on a silica column (HPLC-2). From the yield of dU\* we know the loss in the yield of HMdU labeling, and will correct for that loss in calculating levels of HMdU in WBC DNA. Using dU at 5 pmole level as the reporter molecule, we found that even very low amounts of HMdU (0.01-2 pmole) can be quantitated in a linear fashion (see below).

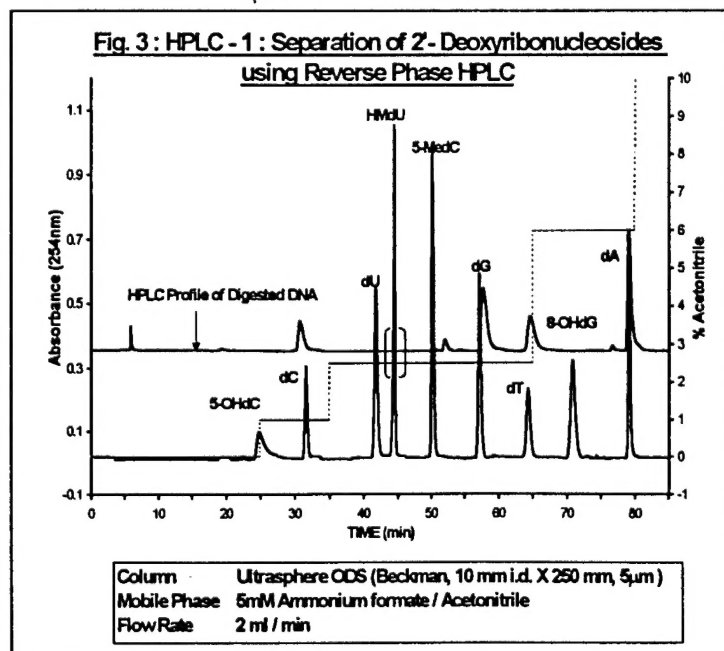
#### 2.e. Limit of Detection (LOD) and Limit of Quantitation (LOQ) of Coumarin-labeled Products

LOD and LOQ for the labeled HMdU and dU products were obtained. It was found that dU\*, HMdU\* I and HMdU\* II were linear in 5 fmole to 2 pmole range. The detection limits for the dU\*, HMdU\* I and HMdU\* II were found to be at level of 0.01 fmole with signal to noise ratio of 10. The standard curve for limit of quantitation of coumarin labeled products is shown in Fig. 2.



#### 2.f. Separation of HMdU from Digested DNA:

In order to validate the post-labeling method before using MCF10-A cells and quantitating the HMdU in blood samples, HMdU has to be isolated from digested DNA of the samples. A reverse-phase HPLC method has been developed to separate HMdU from 2'-deoxyribonucleosides. The HPLC profile of separation of the standard 2'-deoxyribonucleosides overlaid with HPLC profile of digested DNA sample is shown in Fig. 3. In this HPLC method (HPLC-1) HMdU was resolved from other nucleosides with a baseline separation of 10 to 15 min, which minimizes the contamination of the HMdU fraction with other nucleosides. This HPLC-1 will be also used to quantitate normal 2'-deoxynucleosides. For HMdU post-labeling from DNA sample, fraction from 42-46 min of HPLC - 1 will be collected, dried and subjected to HMdU post-labeling for quantitation.



### **3. Significance**

The most significant finding stemming from the studies performed during this time period is that the fluorescent post-labeling reaction occurs on the primary alcohol group of nucleosides. This finding may allow us to extend this post-labeling technique to the quantitation of other modified nucleosides. The quantitation and detection limit of the coumarin labeled HMdU and dU products is in the femtomole range.

### **4. Plan**

This method will be validated using HMdU isolated from MCF10-A cells and it will be utilized to detect and quantitate HMdU isolated from WBC DNA.



### **Key Research Accomplishments to Date:**

- The reagent used for fluorescence labeling of HMdU and described in the Preliminary data was changed from 7-methoxycoumarin-3-carbonylazide to 7-dimethyl-amino coumarin-4-acetic acid (DMACA), which has increased sensitivity and reliability of the post-labeling reaction.
- Post-labeled HMdU, dU & dT products showed that acylation with DMACA occurs on the primary (1°) hydroxy (-OH) groups but not on the secondary hydroxy groups.
- Baseline separation of DMACA-postlabeled HMdU products from reagents was accomplished using normal phase HPLC (HPLC-2); this method can detect and reliably quantitate HMdU in a femtomole range.
- HMdU post-labeling method was optimized by varying all reagents and parameters involved in the reaction.
- A pre-purification method was developed for the postlabeled products to separate them from reagents prior to HPLC analysis.
- Limits of detection (LOD) and limits of quantitation (LOQ) were obtained for labeled dU and HMdU products.
- Reverse-phase HPLC method was optimized for a baseline separation of HMdU from normal 2'-deoxyribonucleosides obtained by enzymatic hydrolysis of DNA (HPLC-1)

### **Reportable Outcome**

This work was presented at 91<sup>st</sup> AACR Annual meeting held in San Francisco, CA (April 1-5, 2000)

- Desai, M., Pelle, E., Sowers, L., and Frenkel, K. Fluorescent post-labeling of 5-hydroxymethyl-2'-deoxyuridine - a sensitive measure of biomarker of oxidative DNA base damage. *Proc. Am. Assoc. Cancer Res.*, **41**:436, 2000

### **Conclusion**

We have developed a sensitive fluorescence post-labeling method for quantitation of HMdU, an oxidized DNA base, which will be utilized for analysis of DNA isolated human WBC, as a measure of oxidative DNA damage. The HMdU-post labeling was accomplished using 7-dimethylamino-coumarin-3-acetic acid in presence of catalyst. The fluorescence labeling occurs at the primary hydroxy (-CH<sub>2</sub>OH) moiety of 2'-deoxyribo-nucleosides as well as 5-CH<sub>2</sub>OH on the base. The method was optimized by varying all reagents and parameters involved in the reaction. The detection limit of the coumarin- labeled nucleoside products was as low as 0.01 fmole, and its quantitation is linear in 2 pmole to 5 fmole range.

Fisher Exact test), compared to mothers who quit smoking during pregnancy. These results imply that an increase in illegitimate V(D)J recombination-mediated alteration, a genetic recombination event associated with childhood malignancies, may be induced *in utero* during pregnancy by maternal exposure to tobacco smoke-derived genotoxins. Supported by NIH RO1 HD33016 to WLB.

**#2776 A MOLECULAR EPIDEMIOLOGIC STUDY OF THE ASSOCIATION BETWEEN ENVIRONMENTAL EXPOSURES TO CARCINOGENS AND DNA DAMAGE AND MUTATION IN MOTHERS AND NEWBORNS.** Frederica P. Perera, J. P. O'Neill, K. Hemminki, W. Jedrychowski, U. Bawle, R. J. Albertini, R. Santella, and R. M. Whyatt, *Columbia Univ, New York, NY, Jagiellonian Univ, Krakow, Poland, Karolinska Inst, Huddinge, Sweden, and Univ of Vermont, Burlington, VT*

This study investigated the potential mutagenic and carcinogenic risks from transplacental exposure to tobacco smoke and air pollutants, including polycyclic aromatic hydrocarbons (PAH) and other aromatic compounds from fossil fuel burning and other combustion processes. We evaluated a battery of biomarkers in cord blood and in maternal blood at delivery among a cohort of 160 mothers and their newborns (total of 320 subjects) in Poland. PAH-DNA adducts in white blood cells (WBC) measured by ELISA were significantly associated with air pollution exposure in both mothers and newborns, as well as with tobacco smoke (active and passive) in the mothers ( $p < 0.05$ ). The same significant associations were not seen between these variables and the total concentration of PAH and other aromatic adducts measured by  $^{32}\text{P}$ -postlabeling. This may be due to the different spectrum of adducts measured by the two methods. Compared to the mothers, the newborns had higher levels of cotinine (an internal dosimeter of tobacco smoke) and both measures of carcinogen-DNA adducts, indicating enhanced susceptibility of the fetus. HPRT mutant frequencies (MF) were elevated in both newborns and mothers compared to other populations studied by this laboratory. In the newborns, HPRT MF was significantly associated with maternal cotinine ( $r = 0.35$ ,  $p = 0.01$ ) and with newborn carcinogen-DNA adducts by postlabeling ( $r = 0.3$ ,  $p = 0.03$ ), providing a molecular link between specific environmental carcinogens and somatic mutation. This study underscores the susceptibility of the fetus and the mutagenic and carcinogenic hazards of fetal exposure to tobacco smoke and airborne carcinogens.

**#2777 SPECTRUM OF CHROMOSOMAL CHANGES DETECTED BY FLUORESCENCE *IN SITU* HYBRIDIZATION IN WORKERS EXPOSED TO BENZENE.** Luoping Zhang, N. Rothman, Y. Wang, W. Guo, R. B. Hayes, G. Li, S. Yin, and M. T. Smith, *Chinese Acad of Preventive Medicine, Beijing, China, National Cancer Inst, Bethesda, MD, and Univ of CA at Berkeley, Berkeley, CA*

Benzene, an established human leukemogen, induces chromosome aberrations in the peripheral blood of exposed workers but little is known about the spectrum of chromosome damage produced. We hypothesized that benzene may cause specific chromosome aberrations related to leukemia in exposed, otherwise healthy workers. We have previously reported data from fluorescence *in situ* hybridization (FISH) analyses of chromosomes 1, 5, 7, 8 and 21 in metaphase spreads from lymphocytes of workers exposed to benzene. Our results showed that chromosomal changes associated with acute myeloid leukemia, including monosomy 5 and 7, trisomy 8, del(5q), del(7q) and t(8;21) were detected at higher levels in exposed workers in comparison with matched controls. We have now performed FISH analysis of five other chromosomes 4, 6, 9, 11 and 22 in the same exposed workers and are in the process of obtaining data for all 22 autosomes. Our new data shows that the deletion of the long-arm of chromosome 6, del(6q), a chromosomal rearrangement commonly found in non-Hodgkins lymphoma, is significantly increased in a dose-dependent fashion in exposed workers ( $p_{\text{trend}} < 0.001$ ). Benzene exposure was not associated, however, with any change in levels of t(9;22), a translocation commonly found in chronic myelogenous leukemia, or in t(4;11) and t(6;11) translocations associated with topoisomerase II inhibitors that form cleavable complexes, such as etoposide. This contrasts with our earlier data which showed highly significant changes in t(8;21) associated with benzene exposure. Our ongoing FISH analysis will soon disclose the full spectrum of chromosome damage produced by benzene. (This work was supported by NIEHS grant RO1 ES06721).

**#2778 FLUORESCENT POST-LABELING OF 5-HYDROXYMETHYL-2'-DEOXYURIDINE - A SENSITIVE MEASURE OF BIOMARKER OF OXIDATIVE DNA BASE DAMAGE.** Mehul K Desai, E. Pelle, L. C. Sowers, and K. Frenkel, *City of Hope National Med Ctr, Duarte, CA, and New York Univ Sch of Medicine, New York, NY*

Recent findings have shown significantly higher levels of 5-hydroxymethyl-2'-deoxyuridine (Hmdu), an oxidized DNA base, in patients with breast cancer and those at high risk to this disease. Hmdu is also proposed to be a biomarker of breast cancer risk. We have developed a simple and sensitive fluorometric HPLC method for Hmdu quantitation, which should be useful in epidemiological studies. This method utilizes 7-dimethylaminocoumarin-3-acetic acid (DMACA) as the fluorescent probe for the pre-column derivatization in anhydrous acetonitrile in the presence of a catalyst at 65°C for 2 hr. Both dU and dT also form fluorescent coumarin products. Reaction with Hmdu resulted in the formation of two baseline separated products. The UV and fluorescence spectra were similar for dT-, dU- and the first of the Hmdu-derived products, as compared to the second Hmdu product. These results suggest that similar to dT and dU products, Hmdu-I is a

result of the reaction on the 5'-CH<sub>2</sub>OH group on the sugar, while Hmdu-II is a product of the reaction with 5-CH<sub>2</sub>OH on the base. The physico-chemical characterization of the products is currently underway. To quantitate Hmdu, cellular DNA is enzymatically digested to nucleosides, which are well separated by HPLC-1. Hmdu containing fractions are concentrated, subjected to coumarin postlabeling and quantitated using normal phase HPLC-2 with fluorescence detection at  $\lambda_{\text{ex}} = 380 \text{ nm}$  and  $\lambda_{\text{em}} = 460 \text{ nm}$ . This method can detect Hmdu at a subpicomole range. [This work was supported in part by AG14587, CA37858, and DAMD17-99-1-9321]

**#2779 INFLUENCE OF POLYMORPHIC GLUTATHIONE S-TRANSFERASE M1 AND P1 ON BENZO(A)PYRENE DIOLEPOXIDE ADDUCTS TO ALBUMIN IN OCCUPATIONALLY EXPOSED POPULATION.** Roberta Pastorelli, A. Cerri, M. Rozio, M. dell'Omo, G. Muzi, G. Abbritti, and L. Airolidi, *Mario Negri Inst for Pharmacological Res, Milano, Italy, Univ of Perugia, Milano, Italy*

We investigated whether the presence of benzo(a)pyrene diolepoxide adducts to albumin (BPDE-SA) among individuals occupationally exposed to polycyclic aromatic hydrocarbons ( $n = 120$ ) could be modulated by the polymorphic genes *GSTM1* and *GSTP1*, involved in the detoxification of BPDE. BPDE-SA adducts were analyzed by GCMS. PCR was used for the characterization of *GSTM1* deletion and *GSTP1* 4 alleles (*GSTP1*\*A (1105, A114), *GSTP1*\*B (1105, A114), *GSTP1*\*C (1105, V114), *GSTP1*\*D (1105, V114)). When analyzed separately, neither *GSTM1* nor *GSTP1* contributed significantly to levels and frequency of adducts. With the two genes analyzed in combination, levels of adducts were not affected. The frequency of detectable adducts significantly doubled only among individuals with *GSTM1* null/*GSTP1*\*A genotype compared to those with *GSTM1* positive/*GSTP1*\*A genotype ( $P = 0.002$ ). In carriers of *GSTP1* variants (*GSTP1*\*, *GSTP1*\*C, *GSTP1*\*D), *GSTM1* genotype did not have any influence on adducts frequency. However, subjects with *GSTM1* null/*GSTP1* variants genotypes had fewer detectable adducts than those who were *GSTM1* null/*GSTP1*\*A. These results suggest that *GSTP1*\*A/*GSTM1* null genotype could be a host factor that increases the presence of BPDE-SA. This is the first *in vivo* study giving some support to the recent *in vitro* finding showing that *GSTP1* variant alleles have greater catalytic efficiency toward BPDE.

**#2780 CYTOCHROME P4502E1 POLYMORPHISM AND GLUTATHIONE S-TRANSFERASE GENOTYPES ARE LINKED TO VINYL CHLORIDE-INDUCED ANGIOSARCOMA.** Deborah Antonino-Green, M. W. Linder, P. Fortwangler, S. Looney, T. E. Geoghegan, and R. Valdes Jr, *Univ of Louisville, Louisville, KY*

In this study we demonstrate a linkage between the *CYP2E1*\*7B allele and glutathione S-transferase (*GSTT1*)-null genotype as risk factors for vinyl chloride (VC)-induced hepatic angiosarcoma (ASL). ASL develops in some individuals following occupational exposure to VC. VC is metabolized by the enzymes cytochrome P4502E1 (*CYP2E1*) and *GST*. We posit that polymorphism in the *CYP2E1* gene or the null genotype of *GSTM1* and *T1* are likely candidates for increasing genetic susceptibility to this cancer. We analyzed the *GSTM1* and *T1*-null versus -native genotypes and known *CYP2E1* polymorphic alleles [*CYP2E1*\*5B ( $C_{1019T}$ ), \*6 ( $G_{480A}$ ), and \*7B ( $G_{-35T}$ )] in DNA collected from individuals with VC-induced ASL. We also compared results with those of disease-free individuals with comparable VC exposure. The frequency of *GSTM1*-positive genotype and *GSTT1*-null genotype are increased two-fold relative to the VC exposed non-diseased subjects. We found no differences in the frequency of the *CYP2E1*\*5B and \*6 alleles, however the *CYP2E1*\*7B allele frequency is increased 4-fold in subjects with ASL 0.200 (95% CI 0.037 to 0.556) versus the non-diseased subjects 0.056 (95% CI 0.010 to 0.186). The \*7B allele is located in the proximal promoter region of the gene suggesting a possible influence on transcriptional regulation. We measured *CYP2E1* mRNA expression in an ASL derived cell-line heterozygous for the \*7B allele and found 4 to 7-fold increased expression relative to cell-lines not containing this polymorphism. These data suggest the *CYP2E1*\*7B allele and *GSTT1*-null genotype are likely risk factors contributing to VC-induced ASL, whereas, *GSTM1* null genotype is protective. This is to our knowledge the first evidence for the role of *CYP2E1*\*7B polymorphism in cancer susceptibility and the first evidence of genetic determinants for increased risk of ASL. (Supported by NIH RO1-ES-08953)

**#2782 XRCC1 GENOTYPE AND NON-MELANOMA SKIN CANCER: RESULTS FROM A CASE-CONTROL STUDY.** Heather H. Nelson, Karl T. Kelsey, Rasheeda Taliaferro, Megan Bronson, and Margaret K. Karagas, *Dartmouth Med Sch, Hanover, NH, and Harvard Sch of Public Health, Boston, MA*

Non-melanoma skin cancer (NMSC) is strongly associated with carcinogen exposures that directly damage DNA, and it is likely that inherited variations in DNA repair capacity may influence NMSC risk. Several common DNA repair polymorphisms have been identified, but their role in disease etiology has not been clearly established. We tested the hypothesis that a genetic polymorphism in the DNA repair gene *XRCC1* is associated with NMSC in a population based case-control study of New Hampshire residents. The *XRCC1* codon 399 Arg→Gln polymorphism was assessed in 202 controls, 244 basal cell carcinoma (BCC) cases, and 148 squamous cell carcinoma (SCC) cases. The prevalence of the homozygous Gln/Gln variant in controls was 21.8%. In both case groups the prevalence of the Gln/Gln genotype was lower than that observed in controls: 13.9% in BCC and 12.8% in SCC. Comparing homozygous genotypes, the crude OR for the variant Gln/Gln genotype and BCC was 0.7 (95% C.I. 0.4-1.1), and 0.6

for SC  
Gln/Gl  
regres:  
0.6 (95  
potent

**#278 STUD**  
Gary E  
and U  
Mut  
gauge  
expos  
sensit  
of the  
and a:  
factor  
is like  
influe  
of fou  
produ  
time p  
sensit  
6 MZ  
0.63,  
sugg  
study  
Supp

**#27: PLAS**  
LAOI  
J. Je  
Baltir  
Ca  
work  
nicol  
canc  
mort  
repo  
cytol  
cerv  
regic  
thos  
Micr  
11 o  
8 (6:  
atio  
pair  
coul  
cyt  
impr  
mer:  
ples

**#2: CEL**  
Dav  
and  
C  
resi  
pro  
so  
dev  
pati  
goe  
tior  
Suc  
tha  
mu  
car  
hos  
DF  
P=  
cor  
TN  
4 (f  
for  
uni